論　文　の　要　旨

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論 文 題 目　Structural and evolutional analysis of *Streptomyces* linear replicons
（放線菌線状レプリコンの構造および進化に関する解析）

Streptomyces is the genus of Gram-positive bacteria with a high G+C content, and is well known for three distinct properties: complex life cycle, the ability to produce secondary metabolites, and the possession of the linear replicon. *Streptomyces* linear chromosome and linear plasmids have common structural features; they possess terminal inverted repeats (TIRs) and terminal proteins bound to the 5' ends of the DNA. The linear chromosomes of *Streptomyces* are very unstable and have a tendency to undergo terminal deletions with a various sizes in each of the species. Terminal deletions generally followed by DNA rearrangements such as amplification, arm replacement, and circularization. In this study, novel chromosomal terminal deletions in *Streptomyces* species were detected, and further analyses were performed to understand unique genome evolution in *Streptomyces* linear replicons.

In this thesis, I describe the general background in Chapter I. Chapter II contains the basic methods and materials used in this study. Chapter III contains the study on the chromosomal deletion in *Streptomyces rochei* 7434AN4 (see **Topic 1**). Chapter IV consists of the analysis of the telomere deletion in *Streptomyces coelicolor* strain No.4 (see **Topic 2**). Chapter V us the general conclusion of two main topics in this thesis.

Topic 1. The *tap-tpg* gene pair on the linear plasmid functions to maintain a linear topology of the chromosome in *Streptomyces rochei.*

Streptomyces rochei 7434AN4, a producer of two antibiotics lankamycin and lankacidin, carries three linear plasmids pSLA2-L (210,614 bp), -M (113,463 bp), and -S (17,526 bp). The nucleotide sequences of these linear plasmids have been determined. Among them, pSLA2-L and -M share the identical 14.6 kb of their right end sequences. In order to detect the chromosomal end of *S. rochei* 7434AN4, the 0.4 kb right-end probe of pSLA2-L was used to analyze several mutants with different plasmid profiles. Southern hybridization indicated that the chromosomal end is located within the 5.5 kb *Bam*HI fragment. However, the plasmidless mutant 2-39 did not show the 5.5 kb *Bam*HI fragment, suggesting that its chromosome had lost the telomeres by circularization. This was confirmed by finding of a fusion sequence in the draft sequence of strain 2-39 obtained by an Illumina GAII sequencer. To verify the loss of telomere sequence in 2-39, new plasmidless mutants were generated from strain 51252 (pSLA2-L^{+}) by protoplast regeneration method. Southern hybridization analysis confirmed the loss of chromosomal telomere as well as pSLA2-L in the newly-synthesized plasmidless mutants YN-P7 and YN-P145. These data clearly show the importance of the linear plasmids. Hence, I further analyzed the candidate genes involved in maintaining linearity of the *S. rochei* chromosome.

S. rochei 7434AN4 carries the intact terminal protein gene (*tpg*) on pSLA2-L and -M, and a truncated gene on the chromosome. There is no *tpg* homolog on pSLA2-S. These findings

suggested that the chromosome does not encode an intact *tpg* gene, and needs either pSLA2-L or pSLA2-M for maintaining its linear topology. To prove this hypothesis, complementation of *tap-tpg* from pSLA2-M (*tapRM-tpgRM*) was carried out in strain 51252 (pSLA2-L$^+$). Plasmid pYN15 carrying the gene pair *tapRM-tpgRM* was transformed into strain 51252, followed by curing of pSLA2-L by protoplast regeneration method in the presence of thiostrepton to retain pYN15. Finally, curing of pYN15 was done by protoplast regeneration without antibiotic supplementation. All the obtained mutants carrying neither pSLA2-L nor pYN15 lost the chromosomal end. These results clearly demonstrate that *tpg-tap* gene pair is essential to maintain the linear topology in *S. rochei* 7434AN4.

Topic 2 (Chapter 4). Chromosomal circularization of the model *Streptomyces* species, *Streptomyces coelicolor* A3(2).

Streptomyces coelicolor A3(2), a model strain of *Streptomyces*, has an 8.7 Mb linear chromosome and produces the blue-pigmented antibiotic actinorhodin (AR). Heat treatment of *S. coelicolor* 1147 provided several mutants, among which strain No. 4 showed the lack of *eshA*, which is important in the production of AR. Since *eshA* located around 130 kb from the right end of the chromosome, the absence of this gene might be related to a loss of terminal region in strain No. 4.

PFGE analysis confirmed the chromosomal end deletion in strain No. 4. Southern hybridization with cosmid 3-14 (nt. 230,501-274,102) and 8-65 (nt. 7,825,517-7,790,510) as probes showed different band pattern between the wild type and mutant. Moreover, both of the probes hybridized to the same band in the mutant No. 4, indicating chromosome circularization. Two sets of PCR primer were designed from each of the cosmid in order to determine the junction region. Cloning and sequence analysis of the amplified DNA detected 6 bp of micro homolog between the right and left end. This result proposed that the circularization occurred by non-homologous end joining (NHEJ) between both deletion ends. It was confirmed that strain No. 4 underwent telomere deletion of 851 kb and 237 kb from the right and left end, respectively.

In this study, I acquired unique insights into dynamic structural change in the *Streptomyces* linear replicons. *S. rochei* 7434AN4 showed telomere deletion concomitant with loss of the linear plasmids. Southern hybridization and sequence analysis of this strain revealed that its chromosome carries no intact *tap-tpg* gene pair. Complementation of *tapRM-tpgRM* (pYN15) and curing experiment of pSLA2-L and pYN15 from the mutants showed that linear plasmids, pSLA2-L and pSLA2-M, function even in maintaining a linear topology of the chromosome in *S. rochei*.

S. coelicolor mutant No. 4 lost a total of 1,088 kb DNA during terminal deletion and circularization. Although mutant No. 4 shows several defective phenotypes, it grows normally. Thus, many genes located in the deleted terminal regions are not essential for survival.

Short homology observed in the fusion junction (9-bp in *S. rochei* strain 2-39 and 6-bp in *S. coelicolor* strain No. 4) suggests that chromosomal circularization occurred by nonhomologous recombination of two deletion ends. This study provided important hints on genome evolution in the *Streptomyces* linear replicons from the structural comparison and studies of their genetic instability.